```
      (FILE 'HOME' ENTERED AT 11:00:31 ON 21 SEP 95)

      FILE 'BIOSIS' ENTERED AT 11:01:08 ON 21 SEP 95
L1           249 COLLAGENASE AND (FAT OR ADIPO?)
L2           146 COLLAGENASE(10A)(FAT OR ADIPO?)
L3             3 COLLAGENASE AND LIPOSUCTION

      FILE 'CA' ENTERED AT 11:21:14 ON 21 SEP 95
L4           213 L1
L5           137 L2
L6             2 L3

      FILE 'MEDLINE' ENTERED AT 11:41:42 ON 21 SEP 95
L7           281 L1
L8           123 L2
L9             4 L3

      FILE 'WPIDS' ENTERED AT 11:53:22 ON 21 SEP 95
L10            8 L1
L11            0 L3

=>
```

356112

L2 ANSWER 1 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 95:309625 BIOSIS
DN 98321925
TI **Collagenase*** lot selection and purification for ***pipase*** tissue digestion.
AU Williams S K; McKenney S; Jarrell B E
CS Section Surgical Res., Dep. Surgery, Univ. Arizona, Health Sci. Cent., 1501 N. Campbell Ave., Tucson, AZ 85724, USA
SO Cell Transplantation 4 (3). 1995. 281-289. ISSN: 0963-6897
LA English
AB Crude Clostridial collagenase (CCC) remains the most widely used enzyme for the digestion of tissues prior to cell isolation and culture. CCC contains numerous components in addition to specific collagenases and proteases. A chronic problem associated with CCC is significant lot variability which occurs with respect to the ability of different lots of CCC to digest tissue. We have evaluated numerous commercially available samples of CCC for their ability to digest human liposuction-derived SC fat. Digestion capacity was evaluated as the ability to release endothelial cells from fat as well as the ability of isolated cells to adhere to tissue culture plastic. A significant variation in digestion efficacy between lots of collagenase was observed. We subsequently purified CCC using a partial purification method with dialysis and centrifugation as well as a complete purification, using liquid chromatography, to remove all nonspecific proteases. While partially purified collagenase retained digestion capacity, pure collagenase exhibited reduced digestion capacity. Maximum digestion was achieved with pure collagenase when trypsin was added. The use of completely purified collagenase with trypsin is advantageous where all components in the enzyme digestion mixture must be known.

L2 ANSWER 12 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 94:267144 BIOSIS
DN 97280144
TI Liposuction-derived human fat used for vascular graft sodding contains endothelial cells and not mesothelial cells as the major cell type.
AU Williams S K; Wang T F; Castrilo R; Jarrell B E
CS Dep. Surgery, University Arizona Health Science Center, Tucson, AZ 85724, USA
SO Journal of Vascular Surgery 19 (5). 1994. 916-923. ISSN: 0741-5214
LA English
AB Purpose: Endothelial cell transplantation has been suggested as a method to improve the patency of prosthetic grafts used for vascular reconstruction. A major technical concern of all cell transplantation studies has been the purity of cells in the primary isolate used for subsequent transplantation. Accordingly we have evaluated the cellular constituents of liposuction-derived human fat with immunocytochemistry and scanning electron microscopy. Methods: Samples of liposuction-derived human fat were processed for immunohistochemistry and subsequently stained for the presence of von Willebrand factor (vWF), alpha-smooth muscle cell actin, cytokeratin (peptide 18), and the endothelial cell-specific marker EN4. We also performed histochemistry studies on the cells derived from this

fat after ***collagenase*** dispersion of the liposuction ***fat*** . Results: Immunohistochemistry revealed that 86.1% of the cells in intact, liposuction-derived fat express vWF, whereas 5.7% of the cells exhibited alpha-smooth muscle cell actin, and 1.0% expressed the mesothelial cell-related antigen, cytokeratin peptide 18. Expression of EN4 was found in 89.6% of the cells counted in intact ***fat*** . After digestion of ***fat*** with ***collagenase*** and centrifugal separation of ***adipocytes*** from vascular and stromal cells, the expression of vWF, alpha-smooth muscle cell actin, and cytokeratin was 77.5%, 5.8%, and 2.1%, respectively. EN4 expression was observed in 74.6% of the isolated cells. Thus most cells present in liposuction-derived fat, even before tissue digestion and cell isolation, were characterized as endothelium. Although other cells common to mesodermally derived tissue were identified (e.g., adipocytes, smooth muscle cells, and mesothelium), they represented a minor fraction of the total cells present. On isolation, the number of cells expressing vWF- and EN4-specific antigens was less than that observed in intact fat. Conclusions: This finding suggests that a portion of cells reacting with antibodies in situ lose vWF and EN4 staining during the isolation procedure. Unlike omentum, liposuction-derived fat predominantly contains adipocytes and endothelial cells. On digestion of liposuction-derived fat and separation of cells, vascular endothelial cells represent the major cellular component.

L2 ANSWER 35 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 92:3924 BIOSIS
DN BA93:3924
TI DIFFERENTIAL MODULATION OF THE ADENYLATE CYCLASE CYCLIC AMP STIMULATORY PATHWAY BY PROTEIN KINASE C ACTIVATION IN RAT ***ADIPOSE*** TISSUE AND ISOLATED ***FAT*** CELLS INFLUENCE OF ***COLLAGENASE*** DIGESTION.
AU DE MAZANCOURT P; DARIMONT C; GIOT J; GIUDICELLI Y
CS LAB. DE BIOCHIMIE DE LA FACULTE DE MEDECINE PARIS-OUEST, HOPITAL DE POISSY, 78303 POISSY CEDEX, FRANCE.
SO BIOCHEM PHARMACOL 42 (9). 1991. 1791-1798. CODEN: BCPCA6 ISSN: 0006-2952
LA English
AB Exposure of rat epididymal fat pad to phorbol 12-myristate 13-acetate (TPA), an activator of protein kinase C, results in an 85% increase in isoproterenol-stimulated cyclic AMP (cAMP) accumulation, an effect which was antagonized by H7, a protein kinase C inhibitor. This promoting action of TPA appears to be related to (i) an increase in the catalytic activity of adenylate cyclase, (ii) an increase in the maximal response of adenylate cyclase to fluoride and guanylimidodiphosphate (GppNHp) with no change in the EC50 value for GppNHp, and (iii) a reduction of the isoproterenol-stimulated low-Km cAMP phosphodiesterase activity present in the 30,000 g pellet of fat pad homogenates. In contrast with fat pads, exposure of isolated rat fat cells to TPA failed to influence their adenylate cyclase response to GppNHp and their cAMP accumulation and lipolysis. However, the other alterations caused by TPA in fat pads were still observed in fat cells. These results suggest that (i) the major alteration responsible for the promoted isoproterenol-stimulated cAMP response observed in fat pads after exposure to TPA is an increased interaction between the .alpha.s subunit of Gs and the catalytic site

by adenylate cyclase and (ii) this increased interaction is dependent on protein kinase C activation and is abolished by collagenase digestion.

L2 ANSWER 49 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 89:11128 BIOSIS
DN BA87:11128
TI CONVERSION OF BIOSYNTHETIC HUMAN PROINSULIN TO PARTIALLY CLEAVED INTERMEDIATES BY ***COLLAGENASE*** PROTEINASES ADSORBED TO ISOLATED RAT ***ADIPOCYTES*** .
AU DOCKWORTH W C; PEAVY D E; HAMEL F G; LIEPNIEKS J; BRUNNER M R; HEINEY R E; FRANK B H
CS VETERANS ADM. MED. CENT., OMAHA, NEBR. 68105, U.S.A.
SO BIOCHEM J 255 (1). 1988. 277-284. CODEN: BIJOAK ISSN: 0306-3275
LA English
AB Studies of the biological activity of proinsulin have resulted in widely varying conclusions. Relative to insulin, the biological activity of proinsulin has been reported from less than 1% to almost 20%. Many of the assays in vitro for the biological potency of proinsulin have utilized isolated rat adipocytes. To examine further the interaction of proinsulin with rat adipocytes, we prepared specifically-labelled proinsulin isomers that were iodinated on tyrosine residues corresponding to the A14, A19, B16 or B26 residue of insulin. These were incubated with rat adipocytes and their metabolism was examined by trichloroacetic acid precipitaiton, by Sephadex G-50 chromatography, and by h.p.l.c. chromatography. By trichloroacetic acid-precipitation assay, there was little or no proinsulin degradation. By G-50 chromatography and subsequent h.p.l.c. analysis, however, we found that the labelled proinsulin isomers were converted rapidly and almost completely to materials which eluted differently on h.p.l.c. from intact proinsulin. This conversion was due primarily to proteolytic activity which adsorbed to the ***fat*** cells from the crude ***collagenase*** used to isolate the cells. Two primary conversion intermediates were found: one with a cleavage at residues 23-24 of proinsulin (the B-chain region of insulin), and one at residues 55-56 in the connecting peptide region. These intermediates had receptor binding properties equivalent to or less than intact proinsulin. These findings show that isolated fat cells can degrade proinsulin to intermediates due to their contamination with proteolytic activity from the collagenase used in their preparation. Thus the previously reported range in biological activities of proinsulin in fat cells may have arisen from such protease contamination. Finally, the present findings demonstrate that a sensitive assay for degradation of hormones is required to examine biological activities in isolated cells.

L2 ANSWER 50 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 88:30621 BIOSIS
DN BA86:23254
TI A METHOD FOR ISOLATING PLASMATIC MEMBRANES FROM RAT ***ADIPOSE*** TISSUE WITHOUT A PRELIMINARY ***COLLAGENASE*** TREATMENT.
AU SOLTAN G G
CS SECT. GERONTOL., ACAD. SCI. B. SSR, MINSK, USSR.
SO VOPR MED KHIM 33 (6). 1987. 132-135. CODEN: VMDKAM ISSN: 0042-8809
LA Russian

AB A rapid procedure has been described for preparation of a relatively pure fraction of plasmatic membranes from ***adipose*** tissue without treatment with ***collagenase*** . Gentle homogenization of rat fatty tissue in a buffered sucrose solution yielded membrane fractions that could be separated from the bulk of contaminating mitochondria and microsomes by a series of differential and isopycnic centrifugations. The preparation obtained was enriched 12-fold with 5'-nucleotidase as compared with the initial homogenate and contained minimal contaminations with mitochondria or elements of endoplasmic reticulum.

L2 ANSWER 74 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 85:261466 BIOSIS
DN BA79:41462
TI OXYGEN CONSUMPTION IN ***COLLAGENASE*** -LIBERATED RAT ***ADIPOCYTES*** IN RELATION TO CELL SIZE AND AGE.
AU AHLGREN P; RADDATZ E; BERGH C-H; KUCERA P; SJOSTROM L
CS DEP. MED. I. SAHLGREN'S HOSP., S413 45 GOTEBORG, SWED.
SO METAB CLIN EXP 33 (10). 1984. 897-900. CODEN: METAAJ ISSN: 0026-0495
LA English
AB Oxygen consumption of ***collagenase*** -liberated rat ***adipocytes*** was measured by 2 different techniques: a microspectrophotometric method using Hb as indicator of respiration and a technique using the oxygen electrode. These 2 completely different techniques gave similar values for oxygen consumption. With the spectrophotometric method, the oxygen consumption of single fat cells was determined. A close positive correlation (r = > 0.90) between oxygen consumption and fat cell size was observed in each tissue examined. With the oxygen electrode technique, oxygen consumption of adipocyte suspensions from young (40 days, 180 g) and old (90 days, 480 g) rats was examined. Fat cells of the suspensions were separated into classes of different size by a flotation technique. A significant positive correlation between fat cell size and oxygen consumption was observed in both young (r = 0.88) and old (r = 0.93) rats. However, the slope was much steeper in young rats. At a cell weight of 0.1 .mu.g the oxygen consumption was 0.364 and 0.086 .mu.l O2/10e6 cells/min-1 in young and old rats, respectively. In the literature, a number of separate metabolic pathways were found to be related positively to fat cell size and negatively to age. These scattered metabolic observations are in agreement with integrated data on energy expenditure as evaluated from oxygen consumption. Estimations of the energy expenditure of adipose tissue indicates that this tissue is responsible for about 1 and 0.5% of the total energy expenditure in young and old rats, respectively.

L2 ANSWER 82 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 84:183894 BIOSIS
DN BA77:16878
TI ISOLATION AND CULTURE OF MICRO VASCULAR ENDOTHELIUM FROM HUMAN ADIPOSE TISSUE.
AU KERN P A; KNEDLER A; ECKEL R H
CS DEP. MED., ENDOCRINOL. DIV., UNIV. COLO. HEALTH SCI. CENT., DENVER, COLO. 80262.
SO J CLIN INVEST 71 (6). 1983. 1822-1829. CODEN: JCINAO ISSN: 0021-9738
LA English
AB The study of human endothelial cells in tissue culture has been

previously limited to umbilical vein, a large vessel source, and microvascular endothelium from human foreskin, spleen and adrenal. Microvascular endothelium cultured from these sources have required matrix-coated culture flasks, tumor-conditioned medium, or 50% human serum for growth and subcultivation. To obtain cultures of microvascular endothelium with less stringent growth requirements, human ***adipose*** tissue was digested with ***collagenase*** and endothelial cells were separated from other stromal elements by sequential filtration and layering cells onto 5% albumin. Using standard medium containing 10% fetal calf serum, these cells grew readily to confluence and survived serial passages. When the cultures were subconfluent, cytoplasmic extensions and a capillary-like morphology were observed. Confluent cultures displayed the cobblestone appearance characteristic of other endothelial preparations. EM demonstrated the presence of characteristic tight junctions and pinocytotic vesicles. Immunofluorescent staining for Factor VIII was positive and cultures contained angiotensin-converting enzyme activity. Cultures of human microvascular endothelium were readily obtained from adipose tissue and required only standard medium with 10% serum for growth and subcultivation. This system can be used to study human endothelial cell biology and may prove useful in the study of pathologic states such as diabetic microvasculopathy and tumor angiogenesis.

L2 ANSWER 84 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 85:027950 BIOSIS
DN BA76:85442
TI COLLAGENASE IN APPROPRIATE CONCENTRATIONS AND NONSPECIFIC PROTEASE DO NOT INTERFERE WITH THE ADRENERGIC RESPONSIVENESS OF ADIPOCYTES.
AU MORLBACHOVA E; JIRICKA Z; MOUREK J
CS INSTITUTE PHARMACOL., FAC. GENERAL MED., 128 00 PRAGUE 2, ALBERTOV 4.
SO PHYSIOL BOHEMOSLOV 31 (6). 1982 (RECD. 1983). 543-548. CODEN: PHBOBQ ISSN: 0369-9463
LA English
AB Adrenergic lipid-mobilization during ontogenesis was studied in trimmed subcutaneous adipose tissue (AT) or its small ***adipocytes*** . The possible negative interference of ***collagenase*** , used for cell separation, was tested. Experiments were carried out on adipocytes from subcutaneous AT of Wistar rats at the age of 14 and 21 days and the results were compared to those obtained in adult animals. Three concentrations of collagenase SEVAC (COL) with 546 PZS activity .cntdot. g-1 (0.5, 1.0 and 2.0 mg/g AT) were used for fat cell isolation. The release of free fatty acids from 1 .times. 106 adipocytes of usual size in each age group served as measure of the lipolytic response. Log concentration-response curves of the isoprenaline (ISO) induced lipid mobilization (IILM) were constructed and quantitatively evaluated. In younger rats the cell yield/1 AT did not change when different ***collagenase*** concentrations were used. In adult animals the ***adipocyte*** yield was the highest when 2 mg/g AT were used. The applied concentrations of collagenase did not affect the ILM. It was constant in adult rats; in 14- and 21-day-old rats irregularities were registered again. Beside experiments with high IILM in all COL-concentration groups (CCG), the absence of the lipolytic activity of ISO in some experiments, especially in 21-day-old rats, could also be observed in all CCG. These discrepancies in adrenergic lipolysis

during ontogenesis cannot be explained by the injurious interference of COL, in the used amount, on adipocyte cell membranes.

L2 ANSWER 104 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 82:111542 BIOSIS
DN BR23:41534
TI EFFECT OF CELL ISOLATION PROCEDURE ON LIPID FILLING OF ADIPOSE TISSUE STROMAL VASCULAR CELLS IN PRIMARY CULTURE.
AU GRUEN R K; FAUST I M; HIRSCH J
CS ROCKEFELLER UNIV., NEW YORK, N.Y. 10021.
SO 66TH ANNUAL MEETING OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY, NEW ORLEANS, LA., USA, APRIL 15-23, 1982. FED PROC 41 (3). 1982. ABSTRACT 695. CODEN: FEPRA7 ISSN: 0014-9446
DT Conference
LA English

L2 ANSWER 105 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 82:136799 BIOSIS
DN BR23:36791
TI ***COLLAGENASE*** ACTION ON THE ADRENERGIC REACTIVITY OF RAT ***ADIPOCYTES*** DURING ONTOGENESIS.
AU MUNZBACHOVA E; MOUREK J
CS DEP. PHARMACOL., FAC. MED., CHARLES UNIV., PRAGUE.
SO MEETING OF THE CZECHOSLOVAK PHYSIOLOGICAL SOCIETY, FEB. 2-4, 1981. PHYSIOL BOHEMOSLOV 30 (5). 1981. 446. CODEN: PHBOBQ ISSN: 0369-9463
DT Conference
LA English

L2 ANSWER 113 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 80:249324 BIOSIS
DN BA70:41820
TI INFLUENCE OF ***ADIPOCYTE*** ISOLATION BY ***COLLAGENASE*** ON PHOSPHO DI ESTERASE ACTIVITY AND LIPOLYSIS IN MAN.
AU ENGFELDT P; ARNER P; OSTMAN J
CS DEP. MED., HUDDINGE HOSP., S-141 86 HUDDINGE, SWED.
SO J LIPID RES 21 (4). 1980. 443-448. CODEN: JLPRAW ISSN: 0022-2275
LA English
AB Phosphodiesterase activity Vmax with low and high Km was, respectively, 10 and 3 times greater in tissue fragments than in ***collagenase*** -isolated ***adipocytes*** obtained from subcutaneous ***fat*** layers in man. The exposure of such tissue fragments to ***collagenase*** of various origins to isolate the ***fat*** cells resulted in a 60-70% inhibition of phosphodiesterase (PDE) activity. Noradrenaline [norepinephrine]- and isoproterenol noradrenaline-induced rates of lipolysis were more rapid in the isolated fat cells than in the tissue fragments. The sensitivity to catecholamines was the same for the 2 tissue preparations. They did not differ in respect to the effect of theophylline, a PDE inhibitor, on the rate of lipolysis. The time curve for cyclic[c]AMP accumulation was significantly higher in the isolated adipocytes than in tissue fragments in the presence of isopropyl noradrenaline. Greater lipolytic response of ***collagenase*** -isolated ***adipocytes*** than of tissue fragments to catecholamines may be attributed, at least in part, to the higher concentration of cAMP resulting from a decrease in PDE activity.

L2 ANSWER 118 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 80:215029 BIOSIS
DN BA70:7525
TI ISOLATION OF PLASMATIC MEMBRANES FROM ***FAT*** CELLS WITHOUT USING ***COLLAGENASE*** .
AU BEZDROBNYI YU V; EVDOKIMOVA N YU
CS KIEV RES. INST. ENDOCRINOL. METAB., MINIST. HEALTH UKR. SSR, KIEV, USSR.
SO VOPR MED KHIM 25 (3). 1979. 354-359. CODEN: VMDKAM ISSN: 0042-8809
LA Russian
AB A method is described for isolation of plasmatic membranes of rat fatty cells immediately from fatty tissue without treatment with collagenase. Homogenization of fatty tissue was carried out in large volumes of buffered sucrose and EDTA at room temperature followed by sucrose density gradient centrifugation. The preparations obtained exhibited high specific activity of the marker enzymes of plasmatic membranes [5'-nucleotidase and K+,Na+-ATPase] and high ability for specific binding of insulin.

L2 ANSWER 133 OF 146 BIOSIS COPYRIGHT 1995 BIOSIS
AN 77:145035 BIOSIS
DN BA63:42899
TI INFLUENCE OF TRYPSIN ON LIPOLYSIS IN HUMAN FAT CELLS COMPARISON WITH RAT ADIPOCYTES.
AU GIUDICELLI Y; PROVIN D; FECQUERY R; NORDMANN R
SO BIOCHIM BIOPHYS ACTA 450 (3). 1976 (RECD 1977) 358-366. CODEN: BBACAQ ISSN: 0006-3002
LA unavailable
AB Trypsin-treated human and rat ***fat*** cells were obtained by digestion of ***adipose*** tissue with ***collagenase*** plus trypsin and their lipolytic response to insulin, catecholamines and dibutyryl cyclic[cAMP were compared with the lipolytic response of human and rat ***fat*** cells isolated with ***collagenase*** only. In both human and rat ***fat*** cells, no significant modification occurred in the intracellular lactate dehydrogenase content and in the basal release of glycerol after trypsination. In rat fat cells, trypsin abolished the antilipolytic effect of insulin but maintained a normal lipolytic response to epinephrine, norepinephrine and isoproterenol. In human fat cells, on the contrary, trypsin failed to modify the antilipolytic effect of insulin, but markedly potentiated the lipolytic response to epinephrine, norepinephrine and isoproterenol. Trypsin also increased the rate of intracellular cAMP accumulation in response to catecholamines. Under these conditions, trypsin-treated human fat cells had a normal response to the lipolytic agent dibutyryl cAMP. Human fat cells apparently differ from the rat ones by the existence in human adipocyte membranes of a trypsin-sensitive component which inhibits the catecholamine induced lipolytic process and which is different from the .alpha. receptors.

L3 ANSWER 1 OF 3 BIOSIS COPYRIGHT 1995 BIOSIS
AN 95:309625 BIOSIS
DN 98023925
TI ***Collagenase*** lot selection and purification for adipose tissue digestion.
AU Williams S K; McKenney S; Jarrell B E
CS Section Surgical Res., Dep. Surgery, Univ. Arizona, Health Sci. Cent., 1501 N. Campbell Ave., Tucson, AZ 85724, USA
SO Cell Transplantation 4 (3). 1995. 281-289. ISSN: 0963-6897
LA English
AB Crude Clostridial ***collagenase*** (CCC) remains the most widely used enzyme for the digestion of tissues prior to cell isolation and culture. CCC contains numerous components in addition to specific collagenases and proteases. A chronic problem associated with CCC is significant lot variability which occurs with respect to the ability of different lots of CCC to digest tissue. We have evaluated numerous commercially available samples of CCC for their ability to digest human ***liposuction*** -derived SC fat. Digestion capacity was evaluated as the ability to release endothelial cells from fat as well as the ability of isolated cells to adhere to tissue culture plastic. A significant variation in digestion efficacy between lots of ***collagenase*** was observed. We subsequently purified CCC using a partial purification method with dialysis and centrifugation as well as a complete purification, using liquid chromatography, to remove all nonspecific proteases. While partially purified ***collagenase*** retained digestion capacity, pure ***collagenase*** exhibited reduced digestion capacity. Maximum digestion was achieved with pure ***collagenase*** when trypsin was added. The use of completely purified ***collagenase*** with trypsin is advantageous where all components in the enzyme digestion mixture must be known.

L3 ANSWER 2 OF 3 BIOSIS COPYRIGHT 1995 BIOSIS
AN 94:26714- BIOSIS
DN 97280144
TI ***Liposuction*** -derived human fat used for vascular graft sodding contains endothelial cells and not mesothelial cells as the major cell type.
AU Williams S K; Wang T F; Castrilo R; Jarrell B E
CS Dep. Surgery, University Arizona Health Science Center, Tucson, AZ 85724, USA
SO Journal of Vascular Surgery 19 (5). 1994. 916-923. ISSN: 0741-5214
LA English
AB Purpose: Endothelial cell transplantation has been suggested as a method to improve the patency of prosthetic grafts used for vascular reconstruction. A major technical concern of all cell transplantation studies has been the purity of cells in the primary isolate used for subsequent transplantation. Accordingly we have evaluated the cellular constituents of ***liposuction*** -derived human fat with immunocytochemistry and scanning electron microscopy. Methods: Samples of ***liposuction*** -derived human fat were processed for immunohistochemistry and subsequently stained for the presence of von Willebrand factor (vWF), alpha-smooth muscle cell actin, cytokeratin (peptide 18), and the endothelial cell-specific marker EN4. We also performed histochemistry studies on the cells derived from this fat

after ***collagenase*** dispersion of the ***liposuction*** fat. Results: Immunohistochemistry revealed that 86.1% of the cells in intact, ***liposuction*** -derived fat express vWF, whereas 5.7% of the cells exhibited alpha-smooth muscle cell actin, and 1.0% expressed the mesothelial cell-related antigen, cytokeratin peptide 18. Expression of EN4 was found in 89.6% of the cells counted in intact fat. After digestion of fat with ***collagenase*** and centrifugal separation of adipocytes from vascular and stromal cells, the expression of vWF, alpha-smooth muscle cell actin, and cytokeratin was 77.5%, 5.8%, and 2.1%, respectively. EN4 expression was observed in 74.6% of the isolated cells. Thus most cells present in ***liposuction*** -derived fat, even before tissue digestion and cell isolation, were characterized as endothelium. Although other cells common to mesodermally derived tissue were identified (e.g., adipocytes, smooth muscle cells, and mesothelium), they represented a minor fraction of the total cells present. On isolation, the number of cells expressing vWF- and EN4-specific antigens was less than that observed in intact fat. Conclusions: This finding suggests that a portion of cells reacting with antibodies in situ lose vWF and EN4 staining during the isolation procedure. Unlike omentum, ***liposuction*** -derived fat predominantly contains adipocytes and endothelial cells. On digestion of ***liposuction*** -derived fat and separation of cells, vascular endothelial cells represent the major cellular component.

L3 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1995 BIOSIS
AN 94:122733 BIOSIS
DN 97135733
TI Microvascular endothelial cell sodding of ePTFE vascular grafts: Improved patency and stability of the cellular lining.
AU Williams S K; Rose D G; Jarrell B E
CS Dep. Surg., Univ. Ariz. Health Sci. Cent., Tucson, AZ 85724, USA
SO Journal of Biomedical Materials Research 28 (2). 1994. 203-212. ISSN: 0021-9304
LA English
AB Small diameter (lt 6 mm) synthetic vascular grafts fail at a clinically unacceptable rate due in large part to their inherent thrombogenicity. The development of a new cellular lining on synthetic vascular grafts would most likely improve the patency rates observed for these grafts in small diameter positions. We have evaluated the use of endothelial cell transplantation to accelerate the formation of a cell lining using microvascular endothelial cells derived from canine falciform ligament fat. This source of fat is histologically similar to human ***liposuction*** fat and was isolated using a ***collagenase*** digestion technique identical to methods used for human ***liposuction*** fat microvessel endothelial cell isolation. The isolated fat endothelial cells were sodded onto 4 mm ePTFE grafts using pressure to force the cells onto the luminal surface. This pressure sodding method permitted cell deposition in less than 3 min. Sodded and control (non-cell-treated) grafts were implanted as interpositional paired grafts using end-to-end anastomoses in the carotid arteries of mixed breed dogs. Each dog therefore received a sodded graft on one side and a control graft on the contralateral side. After 12 weeks of implantation all control grafts were occluded while 86% of the cell-sodded grafts remained patent. Statistical evaluation of the data revealed a

significant improvement in patency of cell sodded grafts (McNemar's chi-2 P = .02). Morphological evaluation of grafts explanted at 5, 12, 26, and 52 weeks following implantation revealed the presence of a cell lining on sodded grafts which remained stable for a period of at least one year. This new cell lining exhibited morphologic characteristics of a nonthrombogenic endothelial cell lining. The development of this new intima, evaluated 5 weeks-1 year after implantation, was not associated with a progressive intimal hyperplasia. From these data we conclude that microvessel endothelial cells derived from canine falciform ligament fat can be rapidly isolated using an operating room compatible method. Cell deposition on synthetic grafts is subsequently accelerated using a pressure sooding technique. A cellular lining forms on the inner surface and is associated with a statistically significant improvement in the function of sodded grafts in a canine carotid artery model.

L5 ANSWER 2 OF 137 CA COPYRIGHT 1995 ACS
AN 122:38911 CA
TI Proteolytic enzyme composition containing collagenase and chymopapain for digesting tissue
IN Lee, Catherine; Hornacek, Cynthia; Dinn, Tan Thanh
PA Baxter International Inc., USA
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
PI WO 9423748 A1 941027
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US4013 940416
PRAI US 93-49613 930416
DT Patent
LA English
AB Proteolytic enzyme compns. and processes for digesting connective tissue are disclosed. The enzyme compns. include collagenase (I), which is essentially free of toxins and non-collagen specific components, and chymopapain (II), which is essentially free of toxins. The enzyme compns. are used for dissocg. microvessel cells from connective tissue. Recovered microvessel cells are incorporated into artificial vessel grafts. A soln. of purified I and II in Plasmolyte electrolyte soln. contg. 0.4% human serum albumin was used to mince liposuctioned fatty tissue at a ratio of 1.2 g tissue to 1 mL of enzyme soln. After incubation of the enzyme-tissue mixt. for 20 min at 37.degree., the adipocytes were sepd. by centrifugation. The no of adipocytes was 8128-13525 cells sized over 7.78 .mu.m as compared to 7944 cells when crude collagenase was used.

L5 ANSWER 48 OF 137 CA COPYRIGHT 1995 ACS
AN 105:57437 CA
TI Isolated adipocytes: an assessment of cell surface changes during their preparation
AU Al-Jafari, Abdulaziz A.; Lee, Stephen R.; Tume, Ronald K.; Cryer, Anthony
CS Dep. Biochem., Univ. Coll., Cardiff, CF1 1XL, UK
SO Cell Biochem. Funct. (1986), 4(3), 169-79
CODEN: CBFUDH; ISSN: 0263-6484
DT Journal
LA English
AB A method is described, based on the detection of adipocyte-specific cell surface antigens, which allows assessment of the relative surface damage incurred by the cells, as exemplified by rat adipocytes, when they are prepd. under a variety of conditions. By using the method it is possible to develop, for any set of reagents, a set of cell isolation conditions (collagenase concn., time of incubation) which will produce minimally damaged cells which exhibit high levels of specific cell surface immunoreactivity. Under certain conditions a recovery from limited surface damage can be achieved, although, when cells are prepd. under more extreme conditions, irreversible surface damage occurs. The surface morphol. of the cells as revealed by SEM. is also clearly affected by the conditions of cell isolation. The method has been used to

define the conditions necessary for the isolation of cells to be used in the study of subtle biochem. responses.

L5 ANSWER 87 OF 137 CA COPYRIGHT 1995 ACS
AN 102:92450 CA
TI Adipocyte isolation for the study of fat metabolism
AU Knyazev, Yu. A.; Korochkin, I. M.; Vakhrusheva, L. L.; Akhmadova, T. M.; Lyubitov, E. N.; Turkina, T. I.; Saoelkina, I. M.; Gel, A. K.
CS Moscow, USSR
SO Biokhim. Identif. Patol. Protsessov Klin. Eksp. (1983), 78-80. Editor(s): Knyazev, Yu. A. Publisher: Vtoroi Mosk. Gos. Med. Inst., Moscow, USSR.
CODEN: 52YZA2
DT Conference
LA Russian
AB The proposed procedure uses ***collagenase*** and is useful for studying the size of ***adipocytes*** during acute myocardial infarction and ischemic heart disease. After initial washing of blood from a 1-g fatty portion, the material was ground, placed in an albumin-contg. (1%) Krebs-Ringer phosphate buffer (pH 7.4) contg. collagenase, and stirred (140-150 cycles/min) for 1 h. After incubation, the suspension was filtered through a nylon filter with pore size 250 .mu.m, and the cells were washed (37.degree.) with the same buffer and finally centrifuged at 1000 rpm. The cell size was measured after staining with acridine orange by using a luminescence microscope. The lipids were detd. in the isolated adipocytes by TLC. Adipocyte sizes in acute myocardial infarction and in ischemic heart disease are discussed.

L5 ANSWER 89 OF 137 CA COPYRIGHT 1995 ACS
AN 93:40964 CA
TI Influence of ***adipocyte*** isolation by ***collagenase*** on phosphodiesterase activity and lipolysis in man
AU Engfeldt, Peter; Arner, Peter; Oestman, Jan
CS Dep. Med., Huddinge Hosp., Huddinge, S-141 86, Swed.
SO J. Lipid Res. (1980), 21(4), 443-8
CODEN: JLPRAW; ISSN: 0022-2275
DT Journal
LA English
AB The max. phosphodiesterase (PDE) activity with low and high Km was, resp., 10- and 3-times greater in tissue fragments than in ***collagenase*** -isolated ***adipocytes*** obtained from s.c. ***fat*** layers in man. The exposure of such tissue fragments to ***collagenase*** of various origins in order to isolate the ***fat*** cells resulted in a 60-70% inhibition of PDE activity. Noradrenaline- and isopropylnoradrenaline-induced rates of lipolysis were more rapid in the isolated fat cells than in the tissue fragments. The sensitivity to catecholamines, however, was the same for the 2 tissue preps. Nor did they differ in respect to the effect of theophylline, a PDE inhibitor, on the rate of lipolysis. The time curve for cAMP accumulation was higher in the isolated adipocytes than in tissue fragments in the presence of isopropylnoradrenaline. The greater lipolytic response of ***collagenase*** -isolated ***adipocytes*** (compared to tissue fragments) to catecholamines may be attributed, at least in some measure, to the higher concn. of cAMP resulting from a decrease

1' PLE activity.

L5 ANSWER 10o OF 137 CA COPYRIGHT 1995 ACS
AN 80:106367 CA
TI Dissociation of lepidopterous tissues with proteolytic enzymes. Comparison of collagenase, trypsin, and pronase
AU Walters, David R.
CS Dep. Biol., Boston Univ., Boston, Mass., USA
SO J. Insect Physiol. (1974), 20(1), 49-54
CODEN: JIPhAF
DT Journal
LA English
AB The fat body and certain other tissues of lepidopterous larvae and diapausing pupae were completely dissocd. by incubation in crude or purified collagenase. Damage to the cells was minimal. Dissocn. with trypsin was incomplete, and Pronase caused extensive damage. All 3 enzymes acted principally by digesting the extracellular connective sheath that envelops the individual lobes of fat body, since the cells at this stage were not intrinsically cohesive. Thus collagen is an important structural component of insect connective membranes.

=>

L6 ANSWER 1 OF 2 CA COPYRIGHT 1995 ACS
AN 120:260223 CA
TI Microvascular endothelial cell sodding of ePTFE vascular grafts: improved patency and stability of the cellular lining
AU Williams, Stuart K.; Rose, Deborah G.; Jarrell, Bruce E.
CS Health Sci. Cent., Univ. Arizona, Tucson, AZ, 85724, USA
SO J. Biomed. Mater. Res. (1994), 28(2), 203-12
CODEN: JBMRBG; ISSN: 0021-9304
DT Journal
LA English
AB Small diam. (<6 mm) synthetic vascular grafts fail at a clin. unacceptable rate due in large part to their inherent thrombogenicity. The development of a new cellular lining on synthetic vascular grafts would most likely improve the patency rates obsd. for these grafts in small diam. positions. The authors have evaluated the use of endothelial cell transplantation to accelerate the formation of a cell lining using microvascular endothelial cells derived from canine falciform ligament fat. This source of fat is histol. similar to human ***liposuction*** fat and was isolated using a ***collagenase*** digestion technique identical to methods used for human ***liposuction*** fat microvessel endothelial cell isolation. The isolated fat endothelial cells were sodded onto 4 mm ePTFE grafts using pressure to force the cells onto the luminal surface. This pressure sodding method permitted cell deposition in less then 3 min. Sodded and control (non-cell-treated) grafts were implanted as interpositional paired grafts using end-to-end anastomoses in the carotid arteries of mixed breed dogs. Each dog therefore received a sodded graft on one side and a control graft on the contralateral side. After 12 wk of implantation all control grafts were occluded while 86% of the cell-sodded grafts remained patent. Statistical evaluation of the data revealed a significant improvement in patency of cell sodded grafts (McNemar's .chi.2 P = .02). Morphol. evaluation of grafts explanted at 5, 12, 26, and 52 wk following implantation revealed the presence of a cell lining on sodded grafts which remained stable for a period of at least one year. This new cell lining exhibited morphol. characteristics of a nonthrombogenic endothelial cell lining. The development of this new intima, evaluated 5 wk-1 yr after implantation, was not assocd. with a progressive intimal hyperplasia. From these data the authors conclude that microvessel endothelial cells derived from canine falciform ligament fat can be rapidly isolated using an operating room compatible method. Cell deposition on synthetic grafts is subsequently accelerated using a pressure sodding technique. A cellular lining forms on the inner surface and is assocd. with a statistically significant improvement in the function of sodded grafts in a canine carotid artery model.

L6 ANSWER 2 OF 2 CA COPYRIGHT 1995 ACS
AN 115:42168 CA
TI Influence of adipose tissue distribution on the biological activity of anorogens
AU Killinger, D. W.; Perel, E.; Daniilescu, D.; Kharlip, L.; Lindsay, W. R. N.
CS Dep. Med., Wellesley Hosp., Toronto, ON, M5S 1A8, Can.

SO Ann. N. Y. Acad. Sci. (1990), 595(Steroid Form., Degrad., Action Peripheral Tissues), 199-211
CODEN: ANYAA9; ISSN: 0077-8923
DT Journal
LA English
AB To establish whether the conversion of androstenedione (A) to estrogens and 5.alpha.-reduced metabolites in human adipose tissue was detd. by the site of origin of the tissue, studies were carried out on adipose stromal cells from different body sites. Adipose tissue was obtained from the breast, omentum, abdomen, lower thigh, upper thigh, buttock, and flank from patients undergoing ***liposuction*** for cosmetic reasons or at surgery. Stromal cells were isolated after incubation of the adipose tissue with ***collagenase*** and were grown in culture using .alpha.-minimal essential medium (MEM) + 15% fetal calf serum. Studies of A metab. were carried out when the cells were between days 4 and 12 in culture. After an 8-h incubation with [3H]A as substrate, estrone (E1), testosterone (T), 5.alpha.-androstanedione (5.alpha.-A-dione), androsterone (AND), and dihydrotestosterone (DHT) were isolated using thin-layer and paper chromatog. The conversion per 1 .times. 10$ cells of A to E1 was >10-fold greater in the upper thigh, buttock, and flank than in the breast, lower thigh, abdomen, or omentum (0.13-3.0 vs. 0.01-0.09%). The formation of 5.alpha.-reduced androgens varied from 0.86-10% and was similar in tissue from different body sites. Cortisol (10-7M) stimulated E1 formation 3-10-fold in cells from all sites, whereas 5.alpha.-reductase activity was either unchanged or increased moderately (up to 2-fold). In cells from the abdomen, omentum, and lower thigh, the formation of 5.alpha.-reduced androgens was >10-fold greater than the formation of E1. In cells from the upper thigh, buttock, and flank, E1 formation was comparable to 5.alpha.-reduced androgen formation. These studies show marked differences in the relative conversion of A to estrogens and 5.alpha.-reduced androgens in adipose stromal cells depending on their site of origin, and they suggest that the distribution of body fat may be a major factor in detg. the biol. effects of secreted androgens.

L7 ANSWER 1 OF 4 MEDLINE
AN 94266932 MEDLINE
TI Microvascular endothelial cell sodding of ePTFE vascular grafts: improved patency and stability of the cellular lining.
AU Williams S K; Rose D G; Jarrell B E
CS Department of Surgery, University of Arizona Health Sciences Center, Tucson 85724.
SO J Biomed Mater Res, (1994 Feb) 28 (2) 203-12.
Journal code: HJD. ISSN: 0021-9304.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9405
AB Small diameter (< 6 mm) synthetic vascular grafts fail at a clinically unacceptable rate due in large part to their inherent thrombogenicity. The development of a new cellular lining on synthetic vascular grafts would most likely improve the patency rates observed for these grafts in small diameter positions. We have evaluated the use of endothelial cell transplantation to accelerate the formation of a cell lining using microvascular endothelial cells derived from canine falciform ligament fat. This source of fat is histologically similar to human ***liposuction*** fat and was isolated using a ***collagenase*** digestion technique identical to methods used for human ***liposuction*** fat microvessel endothelial cell isolation. The isolated fat endothelial cells were sodded onto 4 mm ePTFE grafts using pressure to force the cells onto the luminal surface. This pressure sodding method permitted cell deposition in less then 3 min. Sodded and control (non-cell-treated) grafts were implanted as interpositional paired grafts using end-to-end anastomoses in the carotid arteries of mixed breed dogs. Each dog therefore received a sodded graft on one side and a control graft on the contralateral side. After 12 weeks of implantation all control grafts were occluded while 86% of the cell-sodded grafts remained patent. Statistical evaluation of the data revealed a significant improvement in patency of cell sodded grafts (McNemar's chi 2 P = .02). Morphological evaluation of grafts explanted at 5, 12, 26, and 52 weeks following implantation revealed the presence of a cell lining on sodded grafts which remained stable for a period of at least one year. This new cell lining exhibited morphologic characteristics of a nonthrombogenic endothelial cell lining. The development of this new intima, evaluated 5 weeks-1 year after implantation, was not associated with a progressive intimal hyperplasia. From these data we conclude that microvessel endothelial cells derived from canine falciform ligament fat can be rapidly isolated using an operating room compatible method. Cell deposition on synthetic grafts is subsequently accelerated using a pressure sodding technique. A cellular lining forms on the inner surface and is associated with a statistically significant improvement in the function of sodded grafts in a canine carotid artery model.

L7 ANSWER 2 OF 4 MEDLINE
AN 94223785 MEDLINE

TI ***Liposuction*** -derived human fat used for vascular graft seeding contains endothelial cells and not mesothelial cells as the major cell type.
AU Williams S K; Wang T F; Castrillo R; Jarrell B E
CS Department of Surgery, University of Arizona Health Sciences Center, Tucson 85724.
SO J Vasc Surg, (1994 May) 19 (5) 916-23.
Journal code: KDZ. ISSN: 0741-5214.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9406
AB PURPOSE: Endothelial cell transplantation has been suggested as a method to improve the patency of prosthetic grafts used for vascular reconstruction. A major technical concern of all cell transplantation studies has been the purity of cells in the primary isolate used for subsequent transplantation. Accordingly we have evaluated the cellular constituents of ***liposuction*** -derived human fat with immunocytochemistry and scanning electron microscopy. METHODS: Samples of ***liposuction*** -derived human fat were processed for immunohistochemistry and subsequently stained for the presence of von Willebrand factor (vWF), alpha-smooth muscle cell actin, cytokeratin (peptide 18), and the endothelial cell-specific marker EN4. We also performed histochemistry studies on the cells derived from this fat after ***collagenase*** dispersion of the ***liposuction*** fat. RESULTS: Immunohistochemistry revealed that 86.1% of the cells in intact, ***liposuction*** -derived fat express vWF, whereas 5.7% of the cells exhibited alpha-smooth muscle cell actin, and 1.0% expressed the mesothelial cell-related antigen, cytokeratin peptide 18. Expression of EN4 was found in 89.6% of the cells counted in intact fat. After digestion of fat with ***collagenase*** and centrifugal separation of adipocytes from vascular and stromal cells, the expression of vWF, alpha-smooth muscle cell actin, and cytokeratin was 77.5%, 5.8%, and 2.1%, respectively. EN4 expression was observed in 74.6% of the isolated cells. Thus most cells present in ***liposuction*** -derived fat, even before tissue digestion and cell isolation, were characterized as endothelium. Although other cells common to mesodermally derived tissue were identified (e.g., adipocytes, smooth muscle cells, and mesothelium), they represented a minor fraction of the total cells present. On isolation, the number of cells expressing vWF- and EN4-specific antigens was less than that observed in intact fat. CONCLUSIONS: This finding suggests that a portion of cells reacting with antibodies in situ lose vWF and EN4 staining during the isolation procedure. Unlike omentum, ***liposuction*** -derived fat predominantly contains adipocytes and endothelial cells. On digestion of ***liposuction*** -derived fat and separation of cells, vascular endothelial cells represent the major cellular component.

L9 ANSWER 3 OF 4 MEDLINE
AN 90328676 MEDLINE
TI Influence of adipose tissue distribution on the biological activity of androgens.
AU Kissinger D W; Perel E; Danilescu D; Kharlip L; Lindsay W R

CS Department of Medicine, Wellesley Hospital, University of Toronto, Ontario, Canada.
SO Ann N Y Acad Sci, (1990) 595 199-211.
Journal code: 5NM. ISSN: 0077-8923.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9011
AB To establish whether the conversion of androstenedione (A) to estrogens and 5 alpha-reduced metabolites in human adipose tissue was determined by the site of origin of the tissue, studies were carried out on adipose stromal cells from different body sites. Adipose tissue was obtained from the breast, omentum, abdomen, lower thigh, upper thigh, buttock, and flank from patients undergoing ***liposuction*** for cosmetic reasons or at surgery. Stromal cells were isolated after incubation of the adipose tissue with ***collagenase*** and were grown in culture using alpha-minimal essential medium (MEM) + 15% fetal calf serum. Studies of A metabolism were carried out when the cells were between days 4 and 12 in culture. After an 8-hour incubation with (3H)-A as substrate, estrone (E1), testosterone (T), 5 alpha-androstanedione (5 alpha-A-dione), androsterone (AND), and dihydrotestosterone (DHT) were isolated using thin layer and paper chromatography. The conversion per 1 x 10(6) cells of A of E1 was more than 10-fold greater in the upper thigh, buttock, and flank than in the breast, lower thigh, abdomen, or omentum (0.13-3.0 vs 0.01-0.09%). The formation of 5 alpha-reduced androgens varied from 0.86-10% and was similar in tissue from different body sites. Cortisol (10(-7) M) stimulated E1 formation 3- to 10-fold in cells from all sites, whereas 5 alpha-reductase activity was either unchanged or increased moderately (up to twofold). In cells from the abdomen, omentum, and lower thigh, the formation of 5 alpha-reduced androgens was more than 10-fold greater than the formation of E1. In cells from the upper thigh, buttock, and flank, E1 formation was comparable to 5 alpha-reduced androgen formation. These studies show marked differences in the relative conversion of A to estrogens and 5 alpha-reduced androgens in adipose stromal cells depending on their site of origin, and they suggest that the distribution of body fat may be a major factor in determining the biologic effects of secreted androgens.

L7 ANSWER 4 OF 4 MEDLINE
AN [illegible] MEDLINE
TI Human microvessel endothelial cell isolation and vascular graft seeding in the operating room.
AU Williams S K; Jarrell B E; Rose D G; Fontell J; Kapelan B A; Park P W; Carter Y L
CS Department of Surgery, Thomas Jefferson Medical College, Philadelphia, Pennsylvania 19107.
SO Ann Vasc Surg, (1989 Apr) 3 (2) 146-52.
Journal code: AVS. ISSN: 0890-5096.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 8712

AB We have evaluated multiple factors inherent to an operating room-compatible endothelial cell procurement and sodding procedure. Microvessel endothelial cell isolations have been performed on fat tissue obtained from over 140 patients with a 100% success rate. ***Liposuction*** -derived fat was optimal with respect to cell yield, and isolation time. The devices and equipment used were acceptable to the operating room and the complete cell procurement procedure was successful even in the hands of personnel with minimal training. Fat digestion was achieved using crude clostridial ***collagenase*** , with an average cell yield of 1 x 10(6) microvessel endothelial cells/gm of fat. Evaluation of this procedure with canine fat using an operating room acceptable procedure resulted in a 100% procurement success rate requiring 1.5 hours (+/- .5 hrs) for completion of the fat isolation, and cell isolation procedure. Microvessel EC could subsequently be used in graft seeding or sodding techniques to establish endothelial cell monolayers on vascular grafts. Our results indicate that one person with minimal cell isolation background can reproducibly isolate large quantities of sterile autologous endothelial cells in the operating room for immediate use in endothelial cell seeding/sodding procedures.

L10 ANSWER 1 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 95-196732 [26] WPIDS
DNC C95-091141
TI New acidic, ***fat*** -soluble cpd. TAN-1711 obtd. from Aspergillus - is ***collagenase*** inhibitor and tyrosine kinase inhibitor used to treat tumours and prevent metastasis..
DC B04 D16
PA (TAKE) TAKEDA CHEM IND LTD
CYC 1
PI JP 07112995 A 950502 (9526)* 10 pp
ADT JP 07112995 A JP 93-259749 931018
PRAI JP 93-259749 931018
AB JP07112995 A UPAB: 950705
Compound TAN-1711 (I) or its salts is new. (I) has molecular formula: C18H1408; and specified uv, IR and 13CNMR absorption spectrum maxima, including maxima at 245, 307 and 365 nm, (I) is acidic and lipid-soluble. Also claimed is a method for preparing the compound TAN-1711 by culturing microbes belonging to Aspergillus to accumulate the compound, and separating it. Further claimed are a ***collagenase*** inhibitor and a tyrosine kinase inhibitor both of which comprise the compound TAN-1711.

USE - Since the compound has antitumour and anti-metastasis activities, it is useful for the treatment of malignant tumours.

In an example, Aspergillus sp. FL-36831 was cultured in a slant medium contg. potato dextrose broth (28g), agar (20g), and water (1L) at 28 deg. for 7 days, and further cultured in a seed medium (40ml) (pH6.0) contg. glucose (2%), maltose (3%), soybean powder (1.5%), corn steep liquor (1.0%), peptone (0.5%), yeast essence (0.3%), and NaCl (0.3%) at 28 deg. for 48 hrs. with stirring. The obtd. seed culture soln. (1ml) was then cultured in a fermentation medium (pH7.5) contg. glucose (1.0%), dextrin (4%), soybean powder (0.3%), peptone (0.5%), maltose essence (0.5%), yeast essence (0.2%), FeSO4.7H2O (0.5%), MgSO4.7H2O (0.05%), KH2PO4 (0.1%), and CaCO3 (0.5%) with stirring at 28 deg. for 5 days. The obtd. culture soln. (6L) was adjusted to pH3.0, added with ethyl acetate (8L), stirred for 30min., and filtered. The organic layer was washed with water, and concentrated to obtain an oil substance (36.8g). The substance was dissolved in a mixture of chloroform, methanol, and formic acid (95:2:1, 500ml), eluted with mixtures of chloroform, methanol, and formic acid (98:2:1, 1L, 95:5:1, 2L, 90:10:1, 2L, and 80:20:1, 2L in this order), and fractionated to 500ml portions. The fractions 4-11 were collected, concentrated, and dried to obtain a cruce powder (760mg). The powder was treated with methanol to obtain gray yellow powder of TAN-1711 (580mg). The compound TAN-1711 was found to have a 50% inhibition activity at 0.257 and 10.7 mg/ml against collagens of IV and I types.
Dwg.0/0

L10 ANSWER 2 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 95-100941 [14] WPIDS
DNN N95-079738 DNC C95-045770
TI A soln. for freeze storage of an epidermal cell sheet - contains serum-free glycerol and ethylene glycol.
DC A96 B04 D16 D22 P34

PA (SANA) OTSUKA PHARM CO LTD
CYC 1
PI JP 07023779 A 950127 (9514)* 6 pp
ADT JP 07023779 A JP 93-167822 930707
PRAI JP 93-167822 930707
AB JP07023779 A UPAB: 950412
A soln. for freeze storage of an epidermal cell sheet contg. glycerol and polyethylene glycol but contg. no serum component nor amino acid.

USE/ADVANTAGE - The sheet is used for the treatment of burnt skin. The soln. is effective for freeze storage of an epidermal cell sheet.

In an example, glycerol and PEG 6000 were added to Hanks' soln. respectively to 10 and 5 % to prepare a soln. for freeze storage. A waste skin tissue piece obtained from an operation was washed and sepd. from ***fat*** layer. It was sterilised and washed with a DME medium and again sterilised. It was sepd. to an epidermis and a dermis. The epidermis was dispersed in a DMA medium and cultured and sub-cultured and finally made into a cell sheet. The cell sheet was washed with PBS (-), treated with ***collagenase*** , washed with a DMA medium and then with the soln. prepd. above (aroask D (Taiho Yakuhin Co.) swollen by the soln. was placed on it and they were peeled off together and the soln. was drooped on it and frozen at minus 80 deg. C and the resultant sheet was molten at 37 deg. C and the condition of the molten cell sheet was judged by the adhered cell number. It showed an adhered cell number of 1200 after 35 days storage in liquid nitrogen.
Dwg.0/3

L11 ANSWER 3 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN C94-158940 [43] WPIDS
DNC C94-158940
TI Prodn. of ***fat*** -protein additive for stuffed meat products - uses ground and homogenised heads and feet of dry land poultry as the starting material, and a mixt. of ferments with keratinolytic and ***collagenase*** activity as microbial ferment preparate.
DC D13 D16
IN ANTIPOVA, L V; BUTURLAKINA, L E; SIDELNIKOV, V M
PA (VOTE) VORON TECHN INST
CYC 1
PI SU 1822723 A1 930623 (9443)* RU 6 pp
ADT SU 1822723 A1 SU 91-4905495 910128
PRAI SU 91-4905495 910128
AB SU 1822723 A UPAB: 941216
Use of ground and homogenised heads and feet of dry land poultry as the starting material, and prescribed microbial ferment preparate in prodn. of ***fat*** -protein additive for stuffed meat products, improves its quality. The homogenisate is heated to 80-90 deg.C for 15-30 min., cooled to 40-50 deg.C and ferment preparate added in amounts of 0.8-1.0 wt.%. The mixt. is then fermented for 4.5-5 hours at 40-50 deg.C. The ferment preparate with keratinolytic and ***collagenase*** activity consists of a mixt. of equal amounts of ferments extracted from Streptomyces chromogenes S. gracecus 0832 and Penicillium vortmanii VKM F-2091.

USE - Prodn. is used in meat processing industry.

ADVANTAGE - Quicker process, higher quality product.

Dwg.0/0

L10 ANSWER 4 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 91-275192 [38] WPIDS
CR 88-340436 [52]; 90-356017 [48]; 92-375036 [46]; 92-417284 [51]; 93-109309 [13]; 94-065732 [08]
DNN N91-210214 DNC C91-119239
TI Endothelial cell prod. from digested subcutaneous ***fat*** - prepared in unitary vessel having digestion, waste and isolation chambers.
DC D16 D22 P31 P32 P34
IN BLOMAS, P G; JARRELL, B E; PRAIS, A W; WILLIAMS, S K
PA (BECT) BECTON DICKINSON CO; (UYJE-N) UNIV JEFFERSON THOMAS; (ALCH-I) BLOMAS P G
NP 17
PI EP 446450 A 910918 (9138)*
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
AU 9168680 A 910815 (9140)
JP 2255527 A 910810 (9142)
ZA 9100020 A 911022 (9147)
EP 446450 B1 920930 (9244) 39 pp
AU 648768 B 940505 (9423)
DE 69018246 E 951026 (9548)
ES 2071733 T3 950701 (9533)
ADT EP 446450 A EP 90-124162 901213; ZA 9100020 A ZA 91-20 910102; AU 648768 B AU 91-68680 910104; EP 446450 B1 EP 90-124162 901213; DE 69018246 E DE 90-618246 901213, EP 90-124162 901213; ES 2071733 T3 EP 90-124162 901213
FDT AU 648768 B Previous Publ. AU 9168680; DE 69018246 E Based on EP 446450; ES 2071733 T3 Based on EP 446450
PRAI US 90-477733 900209
AB EP 446450 A UPAB: 950207
Tissue is collected and processed to produce an endothelial cell prod using a vessel comprising a digestion chamber, a waste chamber and an isolation chamber. The tissue is processed within the single vessel under sterile conditions.

Pref the digestion chamber is sepd from the waste chamber by a normally closed check valve. An internal vent extends from the waste chamber to the isolation chamber. The digestion chamber is sepd from the isolation chamber by a screen which retains undigested materials.

USE/ADVANTAGE - An endothelial cell prod is prepd from subcutaneous ***fat*** which is subjected to a controlled ***collagenase*** digestion for 20 mins at 37 deg C. The cell suspension is filtered onto a graft surface which is subsequently used in a surgical grafting process. The entire process requires only about 40 mins. The appts provides a closed, sterile fluid path in which conditions are optimised. The system yields an endothelial cell prod suitable for high density seeding, eg 5,140,000-42,400,000 cells from 50 ccs of ***fat*** . @(22pp Dwg.No.1/15)@

L10 ANSWER 5 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 91-142249 [33] WPIDS

DNC C91-105222
TI New angiogenesis factors derived from rat muscle fibroblast cells - have vascular endothelial cell growing and angiogenesis promoting activity, useful as wound-healing agent.
DC B04
PA (SUNA) SUNTORY LTD
CTC 1
PI JP 03157398 A 910705 (9133)*
ADT JP 03157398 A JP 89-296051 891116
PRAI JP 89-296051 891116
AB JP 03157398 A UPAB: 930928
Angiogenesis factors (polypeptides) show vascular endothelial cell-growing activity, angiogenesis activity, and physicochemical features: (1) m.w.: ca. 30-50 K (SDS electrophoresis), (2) isoelectric point: 6.0-7.0, and (3) heparin-binding capability: none.

USE/ADVANTAGE - Polypeptides are derived from muscle fibroblast cells (MFC) which are released from the rat ***fat*** tissues. Since they show angiogenesis activity as well as vascular endothelial cell-growing activity, they can be applied as a clinically useful wound-healing agent.

In an example, epididymitis ***fat*** tissues of a SD series male rat, were enucleated, washed, cut into thin fragments, and suspended in PBS buffer contg. ***collagenase*** and CaCl2 for dialysis. Resultant tissues were then pipetted, centrifuged, filtered, and subjected to a density gradient fraction. Obtd. intermediate cell layer was added with a soln., and centrifuged for washing. Resultant MFC were cultured in a medium contg. FCS with repeated replacement of a FCS-free medium per week. Collected medium was tested for their endothelial cell-growing activity by utilising calf adrenal capillary endothelial cells, and it was found that the MFC stably secrete angiogenesis factors. Purifcn. of the angiogenesis factors were conducted by subjecting the rat ***fat*** tissue fibroblast cells to an ion-exchange chromatography and a gel filtration.
0/0

L10 ANSWER 6 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 87-182360 [25] WPIDS
DNC C89-080372
TI Decomposing processing residue of chickens - by treatment with protein decomposing enzyme compsn. contg. ***collagenase*** .
DC D12 D16
PA (HONS) YAKULT HONSHA KK
CTC 1
PI JP 01120294 A 890512 (8925)* 4 pp
JP 07022496 B2 950315 (9515) 4 pp
ADT JP 01120294 A JP 87-277387 871104; JP 07022496 B2 JP 87-277387 871104
FDT JP 07022496 B2 Based on JP 01120294
PRAI JP 87-277387 871104
AB JP01120294 A UPAB: 930923
The method is characterised by treating the residue with protein decomposing enzyme compsn. contg. the ***collagenase*** which is not pathogenic obtd. from microbes.

Collagenase originated from Streptomyces is pref.

used and other than ***collagenase*** , trypsin, papain, chymotrypsin, chymopapain, carboxypeptidase, amino peptidase, pronase, etc. can be used. The enzyme compsn. originated from Streptomyces is marketed as ' ***Collagenase*** Yakult' (RTM: HONS). After the decompsn. reaction prod. can be sepd. to oil and ***fat*** , the aq. soln. contg. amino acids peptides and undecomposed solid.

USE/ADVANTAGE - The processing residue of chickens can be decomposed with much higher efficiency and that by using the enzyme compsn. originated Streptomyces, enzymic reaction can be practiced at high temp. and the chicken skin which is difficult to be decomposed, can be decomposed at high temp. in a short time. O/G

L10 ANSWER 7 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 86-246682 [38] WPIDS
CR 89-099201 [13]
DNN N86-184353 DNC C86-106070
TI Measurement of blood flow in animals - by injecting non-radioactively labelled microspheres into blood and counting.
DC D16 P31 P32 P33
IN SEE, J R; SHELL, W E
PA (SEES-N) SEE/SHELL BIOTECHNOLOGY INC; (SHEL-I) SHELL W E; (SEES-N) SEE/SHELL BIOTECHNOLOGY INC
CYC 15
PI EP 194517 A 860917 (8638)* EN 13 pp
R: BE CH DE FR GB IT LI SE
US 4616658 A 861014 (8644)
AU 6654310 A 870910 (8743)
NO 8600628 A 870928 (8743)
JP 62221334 A 870929 (8744)
BR 8602031 A 871222 (8805)
CA 1285219 A 890606 (8927)
IL 78051 A 910415 (9125)#
EP 194517 B1 950201 (9509) EN 16 pp
R: BE CH DE FR GB IT LI SE
DE 3650219 G 950316 (9516)
ADT EP 194517 A EP 86-102544 860227; US 4616658 A US 85-706151 850227; JP 62221334 A JP 86-58446 860318; EP 194517 B1 EP 86-102544 860227; DE 3650219 G DE 86-3650219 860227, EP 86-102544 860227
FDT DE 3650219 G based on EP 194517
PRAI US 85-706151 850227; US 86-899161 860822
RE EP 194517 A LPRE: 950404
Measuring blood flow in an animal comprises (a) non-radioactively labelling microspheres, (b) introducing the microspheres into the blood stream of an experimental animal, (c) determining the number of microspheres in a known vol. of the animal's blood after introduction, (d) sacrificing the animal and recovering a portion of the animal's tissue, (e) determining the number of microspheres present in a known sample size of the tissue and (f) calculating blood flow to the tissue from the results of the determn. The microspheres may be seod. from blood by (a) mixing the blood with an anticoagulating agent, (b) mixing the blood with a haemolysing soln. to break up the red blood cells, (c) removing the haemoglobin from the blood, (d) concentrating the microspheres in the soln., and (e) dispersing the microspheres in the soln. The microspheres are pref.

of size 7-100 micron dia. and may be labelled with a coloured dye or enzyme.

ADVANTAGE - Measurement of blood flow is sensitive and specific, allowing more tests per animal than with radiolabelled spheres. The method is inexpensive and poses no health, safety or disposal problems.

0/0

Dwg.0/0

L10 ANSWER 8 OF 8 WPIDS COPYRIGHT 1995 DERWENT INFORMATION LTD
AN 84-176802 [28] WPIDS
DNC C84-074639
TI Detergent compsn. for pre-sterilisation of medical instruments - contains enzymatic preparation contg. seven proteolytic enzymes.
DC A97 D16 D22 D25 P34
IN ALESHINA, Z F; ALEXEEVA, M I; ANTON, A G; BELINSKY, A L; FEDOROVA, L G; IBRAGIMOVA, R N; LUPOVA, L M
PA (BICU) BIOTECH RES INST
CYC 3
PI US 4456544 A 840626 (8428)* 7 pp
DE 3328882 A 850228 (8510)
JP 60049098 A 850318 (8517)
JP 61032360 B 860726 (8634)
DE 3328882 C 890503 (8918)
ADT US 4456544 A US 83-520813 830805; DE 3328882 A DE 83-3328882 830810; JP 60049098 A JP 83-153359 830824
PRAI US 83-520813 830805
AB US 4456544 A UPAB: 930925
Compsn. (I) comprises (in wt.%) 30-35 Na phosphate, 20-25 Na silicate, 19-22 Na carbonate, 4-6 anionic surfactant (II), 2-4 soap (comprising Na salts of fatty acids), 0.5-2 an enzyme compsn. (III), and the balance Na sulphate.

(III) comprises (in wt.%): 30-60 alkaline protease, 27-45 neutral protease, 0.01-5 elastase, 0.001-4 ***collagenase*** , 0.0001-0.01 leucinaminopeptidase, 0.04-0.15 carboxypeptidase, 0.002-1.5 fibrinolytic enzyme, 0.5-2 lipase, and the balance amylase.

ADVANTAGE - (I) ensures elimination of all protein and ***fat*** contamination from medical instruments and equipment without causing corrosion, using either manual or machine washing at 40-50 deg.C.

0/0

=>

1. 5,441,539, Aug. 15, 1995, Endothelial cell deposition device; Paul S. Mignes, et al., 623/66; 435/1; 623/1, 11 [IMAGE AVAILABLE]

US PAT NO: 5,441,539 [IMAGE AVAILABLE] L3: 1 of 8

ABSTRACT:
Apparatus for depositing a cell product, such as endothelial cell product, in a graft and inserting the graft in a vessel is disclosed. The apparatus preferably comprises a tunneler tube that has a hollow portion for supporting the graft therein, apertures to permit the flow of cell product, and a pointed end cap attached to a distal end of the tunneler tube. The apparatus also has a handle connected to the graft and releasably connected to a proximal end of the tunneler tube that provides an inlet for cell product and outlet in fluid communication with the lumen of the graft. During deposition, the cell product thus flows through the handle, into the graft and exits through the apertures. During insertion, the tunneler tube is manipulated by the handle to enter a vessel, and is then released from the handle and removed to accommodate anastomoses.

2. 5,424,208, Jun. 13, 1995, Method for isolating cells from tissue with a composition containing **collagenase** and chymopapain; Catherine T. Lee, et al., 435/266, 219, 240.2, 243, 267 [IMAGE AVAILABLE]

US PAT NO: 5,424,208 [IMAGE AVAILABLE] L3: 2 of 8

ABSTRACT:
Proteolytic enzyme compositions and processes for digesting connective tissue are provided. The enzyme compositions include **collagenase**, which is essentially free of toxins and non-collagen specific components, and chymopapain, which is essentially free of toxins. The enzyme compositions are used for dissociating microvessel cells from connective tissue. Recovered microvessel cells are incorporated into artificial vessel grafts. The composition preferrably contains **collagenase** having an activity of about 43 nkat/ml to about 51 nkat/ml and chymopapin having an activity of about 0.22 nkat/ml to about 0.44 nkat/ml.

3. 5,422,261, Jun. 6, 1995, Composition containing **collagenase** and chymopapain for hydrolyzing connective tissue to isolate cells; Catherine T. Lee, et al., 435/219; 424/94.2, 94.65, 94.67; 435/212, 240.1 [IMAGE AVAILABLE]

US PAT NO: 5,422,261 [IMAGE AVAILABLE] L3: 3 of 8

ABSTRACT:
Proteolytic enzyme compositions and processes for digesting connective tissue are disclosed. The enzyme compositions include **collagenase**, which is essentially free of toxins and non-collagen specific components, and chymopapain, which is essentially free of toxins. The enzyme compositions are used for dissociating microvessel cells from connective tissue. Recovered microvessel cells are incorporated into artificial vessel grafts. The enzyme compositions preferably contain an aqueous mixture of **collagenase** having an activity of about 43 nkat/ml to about 51 nkat/ml, and chymopapain having an activity of about 0.22 nkat/ml to about 0.44 nkat/ml.

4. 5,409,833, Apr. 25, 1995, Microvessel cell isolation apparatus; Can E. Hu, et al., 435/28c; 422/101, 102, 104; 435/311; 494/36 [IMAGE AVAILABLE]

US PAT NO: 5,409,833 [IMAGE AVAILABLE] L3: 4 of 8

ABSTRACT:
A processing vessel for isolating microvessel endothelial cells from liposuctioned fat tissues includes a fat-receiving basket defined by polyester screen material. Fat tissue removed from a patient by **liposuction** is received into the basket and is rinsed and digested with an enzymatic solution. The freed microvessel endothelial cells from the fat tissues are separated from the fat cells, and from blood cells and other materials which may be present in the basket by centrifuging. A bottom chamber of the processing vessel is configured to define a "pellet" of isolated endothelial cells which may be removed from the processing vessel for deposition on the inner lumenal surface of a synthetic graft which the fat-donor patient is to receive.

5. 5,372,945, Dec. 13, 1994, Device and method for collecting and processing fat tissue and procuring microvessel endothelial cells to produce endothelial cell product; Paul G. Alchas, et al., 435/267; 422/101; 435/271, 288, 311; 494/27, 30, 36 [IMAGE AVAILABLE]

US PAT NO: 5,372,945 [IMAGE AVAILABLE] L3: 5 of 8

ABSTRACT:
Methods and apparatus for collecting and processing tissue to produce an endothelial cell product having a vessel for rinsing, draining, digesting and isolating tissue. The vessel has a rinsing and digesting chamber for containing tissue during processing. An inlet in the rinsing and digesting chamber allows entry of rinsing solution and tissue from a **liposuction** device. A waste chamber in fluid communication with the rinsing and digesting chamber preferably connects with a vacuum source. An isolation chamber is separated from the rinsing and digesting chamber by a screen. An ampule in fluid communication with the isolation chamber includes a pair of ports controlled by valve devices to be selectively in fluid communication with the isolation chamber. After processing, the ampule isolates a pellet of endothelial cells and the valve devices permit the pellet to be in fluid communication with the ports. The method includes providing the vessel, introducing tissue to be processed, orienting the vessel to screen the tissue, introducing an enzyme and agitating to digest the tissue, centrifuging the vessel to transfer the cells from the digested tissue, and isolating the cells for retrieval.

6. 5,311,860, May 17, 1994, Endothelial cell procurement and deposition kit; Paul G. Alchas, et al., 604/319, 320 [IMAGE AVAILABLE]

US PAT NO: 5,311,860 [IMAGE AVAILABLE] L3: 6 of 8

ABSTRACT:
The invention is an endothelial cell procurement and deposition kit for collecting fat from a patient, processing said fat to produce an endothelial cell deposition product, and depositing said product on the surface of a graft, all under sterile conditions established and

maintained within the components of said kit comprised of: fat collection means for collecting subcutaneous fat from a patient; digestion means connectable to said fat collection means to maintain sterility during reception of said fat and for retaining said fat under sterile conditions during rinsing and digestion to produce a digested product; endothelial cell isolation means connectable to said digestion means for maintaining sterile conditions during reception of said digested product and for separating and isolating microvessel endothelial cells from said digested product to produce an endothelial cell product; cell deposition means connectable to said isolation means for maintaining sterile conditions during reception of said endothelial cell product and for depositing said cells on the surface of a graft to be implanted in a patient and facilitating implantation of said endothelial graft into a patient.

7. 5,035,708, Jul. 30, 1991, Endothelial cell procurement and deposition kit; Paul G. Alchas, et al., 623/1; 435/1, 240.21; 600/36; 604/35, 48, 319, 902; 623/12, 15 [IMAGE AVAILABLE]

US PAT NO: 5,035,708 [IMAGE AVAILABLE] L3: 7 of 8

ABSTRACT:
The invention is an endothelial cell procurement and deposition kit for collecting fat from a patient, processing said fat to produce an endothelial cell deposition product, and depositing said product on the surface of a graft, all under sterile conditions established and maintained within the components of said kit comprised of: fat collection means for collecting subcutaneous fat from a patient; digestion means connectable to said fat collection means to maintain sterility during reception of said fat and for retaining said fat under sterile conditions during rinsing and digestion to produce a digested product; endothelial cell isolation means connectable to said digestion means for maintaining sterile conditions during reception of said digested product and for separating and isolating microvessel endothelial cells from said digested product to produce an endothelial cell product; cell deposition means connectable to said isolation means for maintaining sterile conditions during reception of said endothelial cell product and for depositing ssid cells on the surface of a graft to be implanted in a patient and facilitating implantation of said endothelial graft into a patient.

8. 4,883,755, Nov. 28, 1989, Method of reendothelializing vascular linings; R. Anthony Carabasi, et al., 435/240.2; 424/77, 407; 435/1, 2; 514/2, 802; 604/4 [IMAGE AVAILABLE]

US PAT NO: 4,883,755 [IMAGE AVAILABLE] L3: 8 of 8

ABSTRACT:
A method for treating the vascular passage of a patient, damaged by procedures such as an endarterectomy which denude portions of the vascular passages of their endothelial cell linings, is disclosed. In this method, endothelial cells are isolated from the patient's own microvessels, the flow of blood through the patient's damaged vascular passage is interrupted, the endothelial cells isolated from the patient's microvessels are applied to the surface of the denuded portion of the patient's vascular passage in a density sufficient to provide coverage of at least about 50% of said denuded portion, and interruption of blood flow through the vascular passage is maintained for a period of time

sufficient to allow the seeded cells to form an attachment to the vascular lining sufficient to withstand the shear created by resumed blood flow through the vascular passage.
=>